amperometric and the conventional amperometric end point method of analysis of quinidine sulfate powder and quinidine sulfate tablets.

CONCLUSIONS

The data obtained in this investigation indicate that this method of analysis is well suited for the quantitative determination of medicinals whose reaction with bromine is too slow to allow the use of the conventional amperometric end point detection technique with good precision.

The precision and accuracy obtained in this investigation indicate it would be of great value in routine analysis.

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Quantitative Evaluation of Conduction Anesthetics in Albino Mice

By W. R. JONES, T. L. KERLEY, and L. C. WEAVER

Conduction anesthetics were evaluated quantitatively using a test procedure based on the vocalization of mice in response to electrical stimulation.

MICE have been used to study surface anesthesia (1, 2) and in addition have been used to evaluate anesthetics injected directly into the tissues (3–5). A method using mice for the quantitative assessment of conduction anesthetics is described in this report.

EXPERIMENTAL

Male Swiss-Webster albino mice were used as experimental animals. A constant volume (0.05 ml.) of drug solution was injected intramuscularly or subcutaneously using a 27-gauge 0.5-in. needle. Ten minutes after injection, the control foot of each animal was stimulated rapidly and repeatedly until the animal vocalized and then continued to vocalize in response to 10 successive stimulations. Any animal that failed to respond when its control foot was stimulated was eliminated from the test. Approximately 5% of the animals had to be rejected for this reason. The stimulus was an electrical current (100 v. d.c.) delivered by a Grass model S-4 stimulator through bipolar silver electrodes. Immediately after a satisfactory response was established, the foot on the injected side was stimulated 5 times and any animal that failed to vocalize one or more times was classified as being locally anesthetized. Because of the tissue damage resulting from the intense stimuli, each animal was used only once. To facilitate conduction of the electrical current, the foot was moistened with 10% sodium chloride solution just prior to contact with the stimulating electrodes. In a series of preliminary experiments dose response curves were obtained by three methods.

Method A.—A 27-gauge 0.5-in. needle was inserted to its full length posterior to the heel, aimed proximally in such a way that the needle was close and parallel to the femur. The solution (0.05 ml.) was deposited in the muscles posterior to the femur.

Method B.—The needle was first inserted to its full length medially to the heel and parallel to the femur and 0.05 ml. of solution was deposited into muscles on the medial side of the femur. An equal amount of solution was injected also into muscles on the lateral side of the femur.

Method C.—This method consisted of injecting 0.05 ml. of drug solution subcutaneously, medially, and slightly above the heel, infiltrating the space formed by the large tendons posteriorly, and anteriorly by the tibia.

After evaluating the results obtained in the preliminary experiments, an additional series of dose response curves was obtained by *Method C* in order to obtain relative potency values. In each experiment of this series a dose response curve was obtained simultaneously for cocaine hydrochloride and a standard local anesthetic agent. Each relative potency determination was repeated in a separate experiment.

Twenty animals were used to determine each point on a dose response curve except for the preliminary experiment using *Method C*, in which each point was based on 10 animals. A minimum of three different drug concentrations was used to establish each dose response curve in all instances.

RESULTS

The anesthetic doses for 50% of mice (AD_{50}), calculated as described by Bliss (6) from dose response curves obtained by methods *A*, *B*, and C, are presented in Tables I, II, and III. Lambda (λ) values were calculated to serve as a basis for choosing between alternative procedures; the lower the value the greater the precision of the method. Table IV shows the relative potency values calculated (7) from a series of dose response curves obtained by *Method C*. It can be seen that the results of the relative potency determinations were reproducible, although the average λ value was not so low as expected on the basis of the preliminary work. It is interesting to note that the order and magnitude of potency for procaine hydrochloride and dibucaine hydrochloride relative to cocaine hydrochloride did

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TABLE I.—POTENCY OF CONDUCTION ANESTHETICS IN MICE (*Method A*)

Drug	AD_{50}[a]	95% Confidence Limits	λ
Cocaine HCl	3.44	2.21–5.35	0.65
Cocaine HCl	5.43	4.22–6.99	0.32
Procaine HCl	33.13	27.61–41.11	0.26
Procaine HCl	34.49	25.77–46.14	0.35
Dibucaine HCl	5.92	5.00–7.01	0.23
		$\bar{\lambda}$	0.36

[a] Dose expressed as mg./ml

TABLE II.—POTENCY OF CONDUCTION ANESTHETICS IN MICE (*Method B*)

Drug	AD_{50}[a]	95% Confidence Limits	λ
Cocaine HCl	2.07	1.74–2.48	0.19
Procaine HCl	10.91	8.47–14.08	0.30
Dibucaine HCl	0.72	0.48–1.07	0.56
Dyclonine HCl	1.16	0.70–1.91	0.61
		$\bar{\lambda}$	0.42

[a] Dose expressed as mg./ml.

TABLE III.—POTENCY OF CONDUCTION ANESTHETICS IN MICE (*Method C*)

Drug	AD_{50}[a]	95% Confidence Limits	λ
Cocaine HCl	3.29	2.34–4.63	0.18
Procaine HCl	16.20	10.67–24.61	0.20
Dibucaine HCl	3.68	2.78–4.87	0.12
Lidocaine HCl	12.67	7.11–22.56	0.24
Dyclonine HCl	3.86	2.05–7.25	0.26
		$\bar{\lambda}$	0.20

[a] Dose expressed as mg./ml.

not differ materially from the results previously reported for these drugs as infiltration anesthetics (5). It should be noted, however, that the authors were unable to obtain satisfactory dose response curves for dyclonine hydrochloride (except in one of the preliminary experiments) and tetracaine hydrochloride. Dibucaine hydrochloride also did not produce entirely satisfactory results inasmuch as toxicity was encountered at doses that were only 60–75% effective. Even though the variability encountered with the other drugs was greater than one would desire, it is no greater than that which occurs in other similar biological tests (5).

DISCUSSION

A consideration of λ values obtained in the preliminary experiments indicated that methods *A* and *B* were roughly equivalent with respect to precision. However, *Method B* possessed the disadvantage of requiring two injections. Of the three methods, the subcutaneous injection (*Method C*) definitely seemed to be superior because of its lower λ value. Although this apparent advantage was not maintained in subsequent experiments, the greater confidence of the investigators in consistently applying the anesthetic more precisely and easily to the selected area led the authors to adopt this method for future use in our laboratories.

The subcutaneous injection method may be a useful addition to the available procedures for the *in vivo* evaluation of local anesthetics. It possesses the advantage of using inexpensive animals on which other types of anesthetic and toxicity tests can be conducted, thus facilitating the comparison of various activities in the same species. The end point is sharp and objective. The technique is easily learned, rapidly performed, and utilizes equipment which is readily available in most laboratories. Finally, it appears to be satisfactory for screening drugs for local anesthetic activity as well as for their quantitative comparison.

TABLE IV.—BIOASSAY OF CONDUCTION ANESTHETICS IN MICE (*Method C*)

Drug	AD_{50}	Relative Potency[a, b]	λ
Cocaine HCl	2.51	0.18	
Procaine HCl	17.88	(0.13–0.25)	0.28
Cocaine HCl	3.03	0.26	
Procaine HCl	16.54	(0.18–0.36)	0.29
Cocaine HCl	2.20	0.37	
Lidocaine HCl	11.35	(0.25–0.56)	0.33
Cocaine HCl	2.86	0.30	
Lidocaine HCl	9.61	(0.21–0.43)	0.30
Cocaine HCl	2.18	1.04	
Dibucaine HCl	1.74	(0.62–1.74)	0.47
Cocaine HCl	1.73	2.09	
Dibucaine HCl	0.53	(0.91–4.78)	0.75
		λ	0.40

[a] Cocaine HCl used as standard of comparison. [b] Figures in parentheses are 95% confidence limits.

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